520 Rec'd PCT/PTO 14 JAN 2000

FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **1038-1003 MIS:jb**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/462816**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA98/00697	**16 July 1998**	**18 July 1997**

TITLE OF INVENTION

NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

APPLICANT(S) FOR DO/EO/US

Xiaomao Li; Suryprakash Sambhara; and Michel H. Klein

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). -unsigned copy
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☐ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Sequence Listing on comupter-readable and on printed paper forms.
It is hereby stated that the Sequence Listing contained on the computer disk is the same as the Sequence Listing in printed paper form.

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U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/462816	PCT/CA98/00697	1038-1003 MIS:jb

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Search Report has been prepared by the EPO or JPO	**$840.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$670.00**
☐	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$760.00**
☒	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO	**$970.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$970.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	48 - 20 =	28	x $18.00	**$504.00**	
Independent claims	8 - 3 =	5	x $78.00	**$390.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS			=	**$1,864.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
SUBTOTAL			=	**$1,864.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE			=	**$1,864.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED			=	**$1,864.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$1,864.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **19-2253** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Michael I. Stewart
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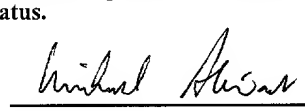


SIGNATURE

Michael I. Stewart

NAME

24,973

REGISTRATION NUMBER

January 13, 2000

DATE

09/462816
430 Rec'd PCT/PTO 14 JAN 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Our Ref: 1038-1003 MIS:jb

In re National Phase of International Application

No.: PCT/CA98/00697

International Filing Date: July 16, 1998

Applicant: Xiaomao Li, et al.

Title: NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

PRELIMINARY AMENDMENT

The Commissioner of Patents
and Trademarks,
Washington, D.C. 20231,
U. S. A.

Dear Sir:

Please amend this application in the following manner:

In the Disclosure:

Before the first line of the specification, add the following:

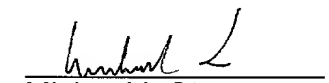
" REFERENCE TO RELATED APPLICATIONS

This application is a national phase application under 35 U.S.C. 371 of PCT/CA98/00697."

REMARKS

The specification has been amended on page 1 to reflect that this application is a U.S. National Phase filing under 35 U.S.C. 371 of PCT/CA98/00697.

Respectfully submitted,



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09/462816
10 Rec'd PCT/PTO 14 JAN 2000
PCT/CA98/00697

TITLE OF INVENTION

NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

FIELD OF INVENTION

The present invention is related to the field of respiratory syncytial virus (RSV) vaccines and is particularly concerned with vaccines comprising nucleic acid sequences encoding the attachment (G) protein of RSV.

BACKGROUND OF INVENTION

Respiratory syncytial virus (RSV), a negative-strand RNA virus belonging to the *Paramyxoviridae* family of viruses, is the major viral pathogen responsible for bronchiolitis and pneumonia in infants and young children (ref. 1 - Throughout this application, various references are referred to in parenthesis to more fully describe the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosures of these references are hereby incorporated by reference into the present disclosure). Acute respiratory tract infections caused by RSV result in approximately 90,000 hospitalizations and 4,500 deaths per year in the United States (ref. 2). Medical care costs due to RSV infection are greater than $340 M annually in the United States alone (ref. 3). There is currently no licensed vaccine against RSV. The main approaches for developing an RSV vaccine have included inactivated virus, live-attenuated viruses and subunit vaccines.

A protective immune response against RSV is thought to require the induction of neutralizing antibodies against the surface fusion (F) and attachment (G) glycoproteins (ref. 4). In addition, cytotoxic T lymphocytes (CTL) responses are involved in viral clearance. The F protein is conserved amongst the RSV A

and B subgroups.

The G protein (33 kDa) of RSV is heavily O-glycosylated giving rise to a glycoprotein of apparent molecular weight of 90 kDa (ref. 5). Two broad subtypes of RS virus have been defined: A and B (ref. 6). The major antigenic differences between these subtypes are found in the G glycoprotein (refs. 3, 7).

The use of RSV proteins as vaccines may have obstacles. Parenterally administered vaccine candidates have so far proven to be poorly immunogenic with regard to the induction of neutralizing antibodies in seronegative chimpanzees. The serum antibody response induced by these antigens may be further diminished in the presence of passively acquired antibodies, such as the transplacentally acquired maternal antibodies which most young infants possess. A subunit vaccine candidate for RSV consisting of purified fusion (F) glycoprotein from RSV infected cell cultures and purified by immunoaffinity or ion-exchange chromatography has been described (ref. 8). Parenteral immunization of seronegative or seropositive chimpanzees with this preparation was performed and three doses of 50 µg were required in seronegative animals to induce an RSV serum neutralizing titre of approximately 1:50. Upon subsequent challenge of these animals with wild-type RSV, no effect of immunization on virus shedding or clinical disease could be detected in the upper respiratory tract. The effect of immunization with this vaccine on virus shedding in the lower respiratory tract was not investigated, although this is the site where the serum antibody induced by parenteral immunization may be expected to have its greatest effect. Safety and immunogenicity studies have been performed in a small number of seropositive individuals. The vaccine was found to be safe in seropositive children and in three

seronegative children (all > 2.4 years of age). The effects of immunization on lower respiratory tract disease could not be determined because of the small number of children immunized. One immunizing dose in seropositive children induced a 4-fold increase in virus neutralizing antibody titres in 40 to 60% of the vaccinees. Thus, insufficient information is available from these small studies to evaluate the efficacy of this vaccine against RSV-induced disease. A further problem facing subunit RSV vaccines is the possibility that inoculation of seronegative subjects with immunogenic preparations might result in disease enhancement. In the 1960's, vaccination of infants with a formalin-inactivated RSV preparation (FI-RSV) resulted in enhanced lung disease upon subsequent exposure to live virus, also referred to as immunopotentiation (refs. 9, 10). These vaccinees developed strong serological responses, but were not protected against infection and some developed severe, occasionally fatal respiratory tract disease upon natural infection. Although precise mechanisms remain unknown, it has been suggested that this form of immune enhancement might reflect either structural alterations of RSV antigens (ref. 11), residual serum and/or cellular contaminants (ref. 12), a specific property of the viral attachment (G) protein (refs. 13,14) or an imbalanced cell-mediated immune response (refs. 13,15). It has been demonstrated that the FI-RSV vaccine induced a TH2-type immune response in mice whereas immunization with live RSV, which does not cause immunopotentiation, elicits a TH1 response (ref.15).

In some studies, the immune response to immunization with a synthetic RSV FG fusion protein resulted in disease enhancement in rodents resembling that induced by a formalin-inactivated RSV vaccine.

Immunization of mice with a recombinant vaccinia virus expressing the RSV G protein resulted in G-specific T cell responses in the lungs which are exclusively recruited from the CD4+T cell sublineage and are strongly Th2-biased. G-specific T cells induce lung haemmorrage, pulmonary neutrophil recruitment (shock lung), intense pulmonary eosinophilia, and sometimes death in the adoptively transferred murine recipients (ref. 14). The association of immunization with disease enhancement using certain vaccine preparations including non-replicating antigens suggests caution in their use as vaccines in seronegative humans.

Live attenuated vaccines against disease caused by RSV may be promising for two main reasons. Firstly, infection by a live vaccine virus induces a balanced immune response comprising mucosal and serum antibodies and cytotoxic T-lymphocytes. Secondly, infection of infants with live attenuated vaccine candidates or naturally acquired wild-type virus is not associated with enhanced disease upon subsequent natural reinfection. It will be challenging to produce live attenuated vaccines that are immunogenic for younger infants who possess maternal virus-neutralizing antibodies and yet are attenuated for seronegative infants greater than or equal to 6 months of age. Attenuated live virus vaccines also have the risks of residual virulence and genetic instability.

Injection of plasmid DNA containing sequences encoding a foreign protein has been shown to result in expression of the foreign protein and the induction of antibody and cytotoxic T-lymphocyte (CTL) responses to the antigen in a number of studies (see, for example, refs. 16, 17, 18). The use of plasmid DNA inoculation to express viral proteins for the purpose of immunization may offer several advantages over the

strategies summarized above. Firstly, DNA encoding a viral antigen can be introduced in the presence of antibody to the virus itself, without loss of potency due to neutralization of virus by the antibodies. Secondly, the antigen expressed *in vivo* should exhibit a native conformation and the appropriate glycosylation. Therefore, the antigen should induce an antibody response similar to that induced by the antigen present in the wild-type virus infection. In contrast, some processes used in purification of proteins can induce conformational changes which may result in the loss of immunogenicity of protective epitopes and possibly immunopotentiation. Thirdly, the expression of proteins from injected plasmid DNAs can be detected *in vivo* for a considerably longer period of time than that in virus-infected cells, and this has the theoretical advantage of prolonged cytotoxic T-cell induction and enhanced antibody responses. Fourthly, *in vivo* expression of antigen may provide protection without the need for an extrinsic adjuvant.

The ability to immunize against disease caused by RSV by administration of a DNA molecule encoding an RSV G protein was unknown before the present invention. In particular, the efficacy of immunization against RSV induced disease using a gene encoding a secreted form of the RSV G protein was unknown. Infection with RSV leads to serious disease. It would be useful and desirable to provide isolated genes encoding RSV G protein and non-replicating vectors, including plasmid vectors, for *in vivo* administration and for use in immunogenic preparations, including vaccines, for protection against disease caused by RSV and for the generation of diagnostic reagents and kits. In particular, it would be desirable to provide vaccines that are immunogenic and protective in humans, including seronegative

infants, that do not cause disease enhancement (immunopotentiation).

SUMMARY OF INVENTION

The present invention relates to a method of immunizing a host against disease caused by respiratory syncytial virus, to non-replicating vectors containing nucleic acid molecules used in immunogenic compositions for such purpose, and to diagnostic procedures utilizing the vectors and nucleic acid molecules. In particular, the present invention is directed towards the provision of nucleic acid vaccines encoding the G protein of respiratory syncytial virus.

In accordance with one aspect of the invention, there is provided an immunogenic composition for *in vivo* administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

The first nucleotide sequence may be that which encodes a full-length RSV G protein. The first nucleotide sequence may comprise the nucleotide sequence shown in Figure 2 (SEQ. ID No: 1) or encode a full length RSV G protein having the amino acid sequence shown in Figure 2 (SEQ. ID no: 2).

Alternatively, the first nucleotide sequence may be that which encodes an RSV G protein from which the transmembrane coding sequence and sequences upstream thereof are absent. The first nucleotide sequence encoding the truncated RSV G protein may comprise the nucleotide sequence shown in Figure 3 (SEQ. ID no: 3) or may comprise a nucleotide sequence encoding the truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID no: 4). The lack of expression of the transmembrane region results in a secreted form of the RSV G protein.

The non-replicating vector may further comprise a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of the first nucleotide sequence. The signal peptide encoding sequence may encode the signal peptide of human tissue plasminogen activator.

The promoter sequence may be an immediate early cytomegalovirus (CMV) promoter. The second nucleotide sequence may comprise the human cytomegalovirus Intron A.

The non-replicating vector generally is a plasmid vector. Plasmid vectors encoding the G protein and included in the immunogenic composition provided by this aspect of the invention may specifically be pXL5 or pXL6, constructed and having their characterizing elements, as seen in Figures 4 or 5, respectively.

In accordance with a further aspect of the present invention, there is provided a method of immunizing a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to the host an effective amount of a non-replicating vector comprising:

a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates

antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host.

The immunization method may be effected to induce a balanced Th1/Th2 immune response.

The present invention also includes a novel method of using a gene encoding respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, to protect a host against disease caused by infection with respiratory syncytial virus, which comprises:

isolating the gene;

operatively linking the gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of the RSV G protein when said vector is introduced into a host to produce an immune response to the RSV G protein, and

introducing the vector into the host.

The procedure provided in accordance with this aspect of the invention may further include the step of:

operatively linking the gene to an immunoprotection enhancing sequence to produce an enhanced immunoprotection by the RSV G protein in the host, preferably by introducing the immunoprotection enhancing sequence between the control sequence and the gene, including introducing immunostimulatory CpG sequences in the vector.

In addition, the present invention includes a method of producing a vaccine for protection of a host

against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding an RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

operatively linking the first nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of the RSV G protein when introduced into a host to produce an immune response to the RSV G protein when expressed *in vivo* from the vector in a host,

operatively linking the first nucleotide sequence to a second nucleotide sequence to increase expression of the RSV G protein *in vivo* from the vector in a host, and

formulating the vector as a vaccine for *in vivo* administration.

The vector may be a plasmid vector selected from pXL5 and pXL6. The invention further includes a vaccine for administration to a host, including a human host, produced by this method.

As noted previously, the vectors provided herein are useful in diagnostic applications. In a further aspect of the invention, therefore, there is provided a method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a non-replicating vector to produce antibodies specific for the RSV G protein, the non-replicating vector comprising a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the

RSV G protein in the host and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;

(b) isolating the RSV G protein-specific antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in the sample and the RSV G protein-specific antibodies; and

(d) determining production of the complexes.

The non-replicating vector employed to elicit the antibodies may be a plasmid vector pXL5 or pXL6.

The invention also includes a diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

(a) a non-replicating vector capable of generating antibodies specific for the RSV G protein when administered to a host, said non-replicating vector comprises a first nucleotide sequence encoding an RSV G protein or an RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in a host, and a second nucleotide sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein *in vivo* from the vector in the host;

(b) isolation means to isolate the RSV G protein specific antibodies;

(c) contacting means to contact the isolated RSV G protein-specific antibodies with the sample to produce a complex comprising any RSV G protein

present in the sample and RSV G protein specific antibodies; and

(d) identifying means to determine production of the complex.

The present invention further is directed to a method for producing antibodies specific for a G protein of a respiratory syncytial virus (RSV) comprising:

(a) immunizing a host with an effective amount of a non-replicating vector to produce RSV G-specific antibodies, said non-replicating vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host; and

(b) isolating the RSV G specific antibodies from the host.

The present invention is also directed to a method for producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV), comprising the steps of:

(a) constructing a vector comprising a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, a promoter sequence operatively coupled to the first nucleotide sequence for expression of the RSV G protein in the host and a second nucleotide

sequence located between the first nucleotide sequence and the promoter sequence to increase expression of the RSV G protein when *in vivo* from the vector in a host;

(b) administering the vector to at least one mouse to produce at least one immunized mouse;

(c) removing B-lymphocytes from the at least one immunized mouse;

(d) fusing the B-lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(e) cloning the hybridomas;

(f) selecting clones which produce anti-RSV G protein antibody;

(g) culturing the anti-RSV G protein antibody-producing clones; and

(h) isolating anti-RSV G protein monoclonal antibodies.

Such monoclonal antibodies may be used to purify RSV G protein from virus.

In this application, the term "RSV G protein" is used to define a full-length RSV G protein, such proteins having variations in their amino acid sequences including those naturally occurring in various strains of RSV, a secreted form of RSV G protein lacking a transmembrane region, as well as functional analogs of the RSV G protein. In this application, a first protein is a "functional analog" of a second protein if the first protein is immunologically related to and/or has the same function as the second protein. The functional analog may be, for example, an immunologically-active fragment of the protein or an immunologically-active substitution, addition or deletion mutant thereof.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following General Description and Examples with reference to the Figures of the accompanying drawings, in which:

Figure 1 illustrates a restriction map of the gene encoding a G protein of respiratory syncytial virus (RSV);

Figure 2 illustrates the nucleotide sequence of a gene encoding a membrane bound form of the G protein of respiratory syncytial virus (SEQ ID No: 1) as well as the amino acid sequence of the RSV G protein encoded thereby (SEQ ID No: 2);

Figure 3 illustrates the nucleotide sequence of a gene encoding the secreted form of the RSV G protein lacking the transmembrane domain (SEQ ID No: 3) as well as the amino acid sequence of a truncated RSV G protein lacking the transmembrane domain encoded thereby (SEQ ID No: 4);

Figure 4 shows the construction of plasmid pXL5 containing a gene encoding a full-length membrane attached form of the RSV G protein and containing the CMV Intron A sequence;

Figure 5 shows the construction of plasmid pXL6 containing a gene encoding a secreted form of the RSV G protein lacking the transmembrane domain and containing the CMV Intron A sequence as well as a nucleotide sequence encoding a signal peptide of the human tissue plasminogen activator (TPA);

Figure 6 shows the nucleotide sequence for the plasmid VR-1012 (SEQ ID No. 5);

Figure 7 shows the nucleotide sequence for the 5' untranslated region and the signal peptide of the human tissue plasminogen activator (TPA)(SEQ. ID no: 6) and

Figure 8 shows the lung cytokine expression profile

in DNA immunized mice after RSV challenge.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention relates generally to polynucleotide, including DNA, immunization to obtain protection against infection by respiratory syncytial virus (RSV) and to diagnostic procedures using particular non-replicating vectors. In the present invention, several recombinant plasmid vectors were constructed to contain a nucleotide sequence encoding an RSV G protein.

The nucleotide sequence of the full length RSV G gene is shown in Figure 2 (SEQ ID No: 1). Certain constructs provided herein include the nucleotide sequence encoding the full-length RSV G (SEQ ID No: 2) protein while others include an RSV G gene modified by deletion of the transmembrane coding sequence and nucleotides upstream thereof (see Figure 3, SEQ ID No: 3), to produce a secreted or truncated RSV G protein lacking the transmembrane domain (SEQ ID No. 4).

The nucleotide sequence encoding the RSV G protein is operatively coupled to a promoter sequence for expression of the encoded RSV G protein *in vivo*. The promoter sequence may be the human immediately early cytomegalovirus (CMV) promoter. This promoter is described in ref. 19. Any other convenient promoter may be used, including constitutive promoters, such as, the Rous Sarcoma Virus LTRs, and inducible promoters, such as the metallothionin promoter, and tissue specific promoters.

The non-replicating vectors provided herein, when administered to an animal in the form of an immunogenic composition with a pharmaceutically-acceptable carrier, effect *in vivo* RSV G protein expression, as demonstrated by an antibody response in the animal to which it is administered. Such antibodies may be used herein in the

detection of RSV protein in a sample, as described in more detail below. The administration of the non-replicating vectors, specifically plasmids pXL5 and pXL6, produced anti-G antibodies, virus neutralizing antibodies, a balanced Th1/Th2 response in the lungs post viral challenge and conferred protection in mice against live RSV infection, as seen from the Examples below.

The recombinant vector also may include a second nucleotide sequence located adjacent the RSV G protein encoding nucleotide sequence to enhance the immunoprotective ability of the RSV G protein when expressed *in vivo* in a host. Such enhancement may be provided by increased *in vivo* expression, for example, by increased mRNA stability, enhanced transcription and/or translation. This additional sequence generally is located between the promoter sequence and the RSV G protein-encoding sequence. This enhancement sequence may comprise the immediate early cytomegalovirus Intron A sequence.

The non-replicating vector provided herein may also comprise an additional nucleotide sequence encoding a further antigen from RSV, an antigen from at least one other pathogen or at least one immunomodulating agent, such as a cytokine. Such vector may contain the additional nucleotide sequence in a chimeric or a bicistronic structure. Alternatively, vectors containing the additional nucleotide sequence may be separately constructed and coadministered to a host, along with the non-replicating vectors provided herein.

The non-replicating vector may further comprise a nucleotide sequence encoding a heterologous viral or eukaryotic signal peptide, such as the human tissue plasminogen activator (TPA) signal peptide, in place of the endogenous signal peptide for the truncated RSV G

protein. Such nucleotide sequence may be located immediately upstream of the RSV G encoding sequence in the vector.

The immunogenicity of the non-replicating DNA vectors may be enhanced by inserting immunostimulatory CpG sequences in the vector.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of RSV infections. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the RSV G genes and vectors as disclosed herein. The vaccine elicits an immune response in an animal which includes the production of anti-RSV G antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid may be prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 9324640, ref. 20) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435

describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co- glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosae. The delivery vehicle may additionally contain an absorption enhancer.

The RSV G gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and

combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the RSV G protein and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 µg

to about 2 mg of the RSV G gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as

adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding an G protein of RSV may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The immunogenicity of the non-replicating vector may be enhanced by coadministering plasmid DNA vectors expressing cytokines or chemokines or by coexpressing such molecules in a bis-cistronic or fusion construct.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 21) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 22) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

2. Immunoassays

The RSV G genes and vectors of the present invention are useful as immunogens for the generation of anti-G antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or

procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the RSV G protein. These RSV G-specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove unadsorbed antibodies, a non-specific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of non-specific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound RSV G specific antibodies, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

BIOLOGICAL MATERIALS

Certain plasmids that contain the gene encoding the RSV G protein and referred to herein have been deposited

with the American Type Culture Collection (ATCC) located at 12301 Parklawn Drive, Rockville, Maryland, 20852, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

Samples of the deposited plasmids will become available to the public upon grant of a patent based upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Samples of the deposited plasmids will be replaced if the depository is unable to dispense viable samples. The invention described and claimed herein is not to be limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention. Any equivalent or similar plasmids that encode similar or equivalent antigens as described in this application are within the scope of the invention.

Plasmid	ATCC Designation	Date Deposited
pXL5	209143	July 16, 1997
pXL6	209144	July 16, 1997

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein biochemistry, and immunology used but not explicitly described in this disclosure and these Examples are

amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example describes the construction of vectors containing the RSV G gene.

Figure 1 shows a restriction map of the gene encoding the G protein of respiratory syncytial virus and Figure 2 shows the nucleotide sequence of the gene encoding the full-length RSV G protein (SEQ ID No: 1) and the deduced amino acid sequence (SEQ ID No: 2). Figure 3 shows the gene encoding the secreted RSV G protein (SEQ ID No: 3) and the deduced amino acid sequence (SEQ ID No: 4).

Plasmid pXL5 (Figure 4) was prepared for the expression of the full-length RSV G protein as follows:

A recombinant Bluescript plasmid (RSV G12) containing the cDNA encoding the full-length G protein of a clinical RSV isolate (subgroup A) was used to construct vectors for RSV DNA-G immunization. RSV G12 was digested with AflIII and EcoRI and filled-in with the Klenow subunit of DNA polymerase. The resulting 1.23 kb fragment containing the coding sequence for the full-length G protein was gel-purified and ligated to VR-1012 (Vical) (Figure 6) previously linearized with EcoRV. This procedure placed the RSV G cDNA downstream of the immediate-early cytomegalovirus (CMV) promoter and Intron A sequences of human cytomegalovirus (CMV) and upstream of the bovine growth hormone (BGH) poly-A site. The junctions of the cDNA fragments in the plasmid construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL5.

Plasmid pXL6 (Figure 5) was prepared for the expression of a secretory RSV G protein as follows:

RSV G12 was digested with EcoRI, filled-in with Klenow and digested again with BamHI. The BamHI

cleavage resulted in the generation of a cDNA fragment encoding a RSV G protein with N-terminal truncation. This DNA segment was gel-purified and ligated in the presence of a pair of 11 mer oligodeoxynucleotides (5'GATCCACTCAG 3') (SEQ ID no: 7)

3' GTGAGTCCTAG 5' (SEQ ID no: 8)

to VR-1020 (Vical) previously digested with BglII, filled in with Klenow, digested again with BamHI and gel-purified. This procedure placed the truncated RSV G cDNA (lacking the coding region for the N-terminal 91 amino acid residues including the transmembrane domain) downstream of the immediate-early CMV promoter and Intron A sequences of human CMV and upstream of the BGH poly-A site. In addition, there was the introduction of approximately 100 bp of 5' untranslated region and the coding sequence for the signal peptide of human plasminogen activator protein (Figure 7) fused in frame to the N-terminus of the RSV G protein coding sequence downstream of the CMV promoter/Intron A sequences. The junctions of the cDNA fragments in the plasmid construct were confirmed by sequencing analysis. The resulting plasmid was designated pXL6.

Example 2

This Example describes the immunization of mice. Mice are susceptible to infection by RSV as described in ref. 24.

Plasmid DNA was purified through double CsCl centrifugations. For intramuscular (i.m.) immunization, tibialis anterior muscles of BALB/c mice (male, 6 to 8 week old) (Jackson Lab., Bar Harbor, ME, USA) were bilaterally injected with 2 x 50µg (1µg/µL in PBS) of either pXL5, pXL6 or V-1012. Five days prior to DNA injection, the muscles were treated with 2 x 50µL (10µM in PBS) of cardiotoxin (Latoxan, France) to increase DNA uptake and enhance immune responses, as reported by

Davis et al (ref. 23). The animals were boosted with the same dose of plasmid DNA 6 weeks and 13 weeks later, respectively. For intradermal (i.d.) immunization, 100μg of the plasmid DNA (2μg/μL in PBS) of were injected at the base of the tail and boosted 6 weeks and 13 weeks later, respectively. Mice in the positive control group were immunized intranasally (i.n.) with 10^6 plaque forming units (pfu) of a clinical RSV strain of the A2 subtype grown in Hep2 cells kindly provided by Dr. B. Graham (ref. 24).

Four weeks after the third immunization, mice were challenged intranasally with 10^6 pfu of the RSV A2 strain. Lungs were asceptically removed 4 days later, weighed and homogenized in 2 mL of complete culture medium (ref. 25). The number of pfu in lung homogenates was determined in duplicate as previously described (ref. 26) using vaccine-quality Vero cells.

Example 3

This Example describes the immunogenicity and protection by polynucleotide immunization.

Antisera obtained from immunized mice were analyzed for anti-RSV G IgG antibody titres using specific enzyme-linked immunosorbent assay (ELISA) and for RSV-specific plaque-reduction titres. ELISAs were performed using 96-well plates coated with immunoaffinity-purified RSV G protein (50 ng/mL)and 2-fold serial dilutions of immune sera. A goat anti-mouse IgG antibody conjugated to alkaline phosphatase (Jackson ImmunoRes., Mississauga, Ontario, Canada) was used as secondary antibody. Plaque reduction titres were determined according to Prince et al (ref. 26) using vaccine-quality Vero cells. Four-fold serial dilutions of immune sera were incubated with 50 pfu of the RSV Long strain (ATCC) in culture medium at 37°C for 1 hr in the presence of 5% CO_2 and the mixtures were used to infect

Vero cells. Plaques were fixed with 80% methanol and developed 5 days later using a mouse anti-RSV F monoclonal IgG1 antibody and donkey anti-mouse IgG antibody conjugated to peroxidase (Jackson ImmunoRes., Mississauga, Ontario, Canada). The RSV-specific plaque reduction titre was defined as the dilution of serum sample yielding 60% reduction in plaque number. Both ELISA and plaque reduction assays were performed in duplicate and data are expressed as the means of two determinations.

The results obtained are reproduced in Tables I and II below:

Table I. Immunogenicity of DNA-G in BALB/c Mice

Immunogen *Titre*	*Anti-RSV G IgG Titre (Log 2(titre/100)*			*RSV-Specific Plaque Reduction*
	6 weeks	*10 weeks*	*17 weeks*	*(Log 2 titre) 17 weeks*
VR-1012 (i.m.)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
pXL5 (i.m.)	3.10 ± 2.77	9.70 ± 1.06	8.60 ± 1.17	5.40 ± 1.65
pXL6 (i.m.)	5.78 ± 1.20	9.30 ± 0.82	8.89 ± 1.54	7.26 ± 0.82
pXL5 (i.d.)	1.50 ± 1.27	8.60 ± 1.43	8.30 ± 1.25	7.92 ± 0.59
pXL6 (i.d.)	3.70 ± 1.25	10.30 ± 1.06	9.44 ± 1.24	6.92 ± 0.94
RSV (i.n.)	6.83 ± 0.41	9.67 ± 0.52	9.83 ± 0.41	11.80 ± 0.08

Table II. Immunoprotective Ability of DNA-G in BALB/c Mice

Immunogen	*No. Mice*	*Mean Virus Lung Titre* (pfu/g lung) (Log 10 ± SD)*	*No. Fully Protected Mice#*
VR-1012 (i.m.)	6	4.81 ± 0.01	0
pXL5 (i.m.)	6	0.29 ± 0.90	5
pXL6 (i.m.)	6	0.40 ± 1.20	5
pXL5 (i.d.)	6	0.30 ± 1.10	5
pXL6 (i.d.)	6	0.29 ± 0.90	5
RSV (i.n.).	6	0.00 ± 0.00	6

*Sensitivity of the assay: $10^{1.96}$ pfu/g lung.

The term, fully protected mice, refers to animals with no detectable RSV in the lungs 4 days post viral challenge.

As seen in Table I, plasmids pXL5 and pXL6 were found to be immunogenic following either i.m. or i.d. immunization producing anti-G antibodies and virus neutralizing antibodies. In addition, as seen in Table II, the plasmids pXL5 and pXL6 protected immunized mice against primary RSV infection of the lower respiratory tract. The control vector produced no immune response and did not confer protection.

Example 4

This Example describes the determination of the local lung cytokine expression profile in mice immunized with pXL5 and pXL6 after RSV challenge.

BALB/c mice were immunized at 0 and 6 weeks with 100μg of pXL5 and 6, prepared as described in Example 1, and challenged with RSV i.n. at 10 weeks. Control animals were immunized with placebo PI-RSV and live RSV and challenged with RSV according to the same protocol. In addition, animals were immunized with pXL2, as described in copending United States Patent Application no. 08/476,397 filed June 7, 1995 (WO 96/40945) and challenged with RSV, also following the same protocol. Four days post viral challenge, lungs were removed from immunized mice and immediately frozen in liquid nitrogen. Total RNA was prepared from lungs homogenized in TRIzol/β-mercaptoethanol by chloroform extraction and isopropanol precipitation. Reverse transcriptase-polymerase chain reaction (RT-PCR) was then carried out on the RNA samples using either IL-4, IL-5 or IFN-γ specific primers from CloneTech. The amplified products were then liquid-hybridized to cytokine-specific ^{32}P-labeled probes from CloneTech, resolved on 5% polyacrylamide gels and quantitated by scanning of the radioactive signals in the gels. Three mouse lungs were removed from each treatment group and analyzed for lung cytokine expression for a minimum of two times. The

data is presented in Figure 8 and represents the means and standard deviations of these determinations.

As may be seen from the data presented in Figure 8:

1. Immunization with live RSV intranasally (i.n.) resulted in a balanced cytokine profile (IFN-γ, IL-4 and IL-5), whereas that with FI-RSV intramuscularly (i.m.) resulted in a Th2 predominance (elevated IL-4 and IL-5). These results are similar to those reported in the literature.

2. Immunization with pXL5 or pXL6 via either the i.m. or intradermal (i.d.) route gave rise to a balanced cytokine profile similar to that with live RSV immunization.

3. The magnitude of the cytokine responses with i.m. pXL6 (RSV G) and pXL2 (RSV F) immunization using the construct expressing a secretory form of the protein (SEC) is significantly higher than that with live RSV immunization.

4. The magnitude of the cytokine response with pXL5 immunization using constructs expressing a full-length membrane-associated RSV G protein (MA) and i.d. pXL6 was somewhat higher than that with live RSV immunization.

5. The balanced local cytokine response observed with DNA-G immunization contrasts with that reported by Openshaw et al (ref. 13). Using a recombinant vaccinia virus expressing the G protein, these investigators reported a local Th2 response by analysis of bronchoalveolar lavage. The results herein, which were obtained through a monogenic approach, indicate that the Th2 response is not necessarily an intrinsic property of the G protein.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention provides certain novel non-replicating vectors containing genes encoding RSV G proteins, methods of immunization using such vectors and methods of diagnosis using such vectors. Modifications are possible within the scope of this invention.

SUBSTITUTE SHEET (RULE 26)

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CLAIMS

What we claim is:

1. An immunogenic composition for *in vivo* administration to a host for the generation in the host of protective antibodies to respiratory syncytial virus (RSV) G protein, comprising a vector that will not replicate when introduced into the host to be protected comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host, and

a pharmaceutically-acceptable carrier therefor.

2. The composition of claim 1 wherein said first nucleotide sequence encodes a full-length RSV G protein.

3. The composition of claim 2 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

4. The composition of claim 2 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein having the amino acid sequence shown in Figure 2 (SEQ ID NO:2).

5. The composition of claim 1 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.

6. The composition of claim 5 wherein said vector further comprises a heterologous signal peptide encoding nucleotide sequence immediately upstream of the 5'-terminus of said first nucleotide sequence.

7. The composition of claim 6 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

8. The composition of claim 5 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).

9. The composition of claim 5 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a truncated RSV G protein having the amino acid sequence shown in Figure 3 (SEQ ID NO:4).

10. The composition of claim 1 wherein said promoter sequence is an immediate early cytomegalovirus promoter.

11. The composition of claim 1 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.

12. The composition of claim 1 wherein the vector is a plasmid vector.

13. The composition of claim 12 wherein the plasmid vector is pXL5 as shown in Figure 4.

14. The composition of claim 12 wherein the plasmid vector is pXL6 as shown in Figure 5.

15. A method of immunizing a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises administering to said host an effective amount of a vector that will not replicate when introduced into the host to be protected comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host.

16. The method of claim 15 wherein said first nucleotide sequence encodes a full-length RSV G protein.

17. The method of claim 16 wherein said nucleotide sequence comprises the nucleotide sequence shown in Figure 2 (SEQ ID NO:1).

18. The method of claim 16 wherein said first nucleotide sequence comprises the nucleotide sequence encoding a full length RSV G protein shown in Figure 2 (SEQ ID NO:2).

19. The method of claim 15 wherein said first nucleotide sequence encodes a RSV G protein from which the transmembrane coding sequence and sequences upstream thereto are absent.

20. The method of claim 19 wherein said vector further comprises a heterologous signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.

21. The method of claim 20 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

22. The method of claim 19 wherein said first nucleotide sequence comprises the nucleotide sequence shown in Figure 3 (SEQ ID NO:3).

23. The method of claim 19 wherein said first nucleotide sequence comprises a nucleotide sequence encoding a transverse RSV G protein shown in Figure 3 (SEQ ID NO:4).

24. The method of claim 15 wherein said promoter sequence is an immediate early cytomegalovirus promoter.

25. The method of claim 15 wherein said second nucleotide sequence is the human cytomegalovirus Intron A.

26. The method of claim 15 wherein the vector is a plasmid vector.

27. The method of claim 26 wherein said plasmid vector is pXL5 as shown in Figure 4.

28. The method of claim 26 wherein said vector is pXL6 as shown in Figure 5.

29. The method of claim 15 wherein a balanced Th1/Th2 immune response is induced.

30. A method of using a gene encoding a respiratory syncytial virus (RSV) G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein, to produce an immune response in a host, which comprises:

isolating said gene,

operatively linking said gene to at least one control sequence to produce a vector that will not replicate when introduced into the host to be protected, said control sequence directing expression of said RSV G protein when introduced into a host to produce an immune response to said RSV G protein, and

introducing said vector into a host.

31. The method of claim 30 wherein said gene encoding a RSV G protein encodes a full length RSV G protein.

32. The method of claim 30 wherein said gene encoding a RSV G protein encodes a RSV G protein lacking the transmembrane domain and sequences upstream thereto.

33. The method of claim 32 wherein said vector further comprises a signal peptide encoding nucleotide sequences immediately upstream of the 5'-terminus of said first nucleotide sequence.

34. The method of claim 33 wherein said signal peptide encoding sequence encodes the signal peptide for human tissue plasminogen activator.

35. The method of claim 30 wherein said at least one control sequence comprises the immediate early cytomegalovirus promoter.

36. The method of claim 35 including the step of:

operatively linking said gene to an immunoprotection enhancing sequence to produce an enhanced immunoprotection to said RSV G protein in said host.

37. The method of claim 36 wherein said immunoprotection enhancing sequence is introduced into said vector between said control sequence and said gene.

38. The method of claim 37 wherein said immunoprotection enhancing sequence is the human cytomegalovirus Intron A.

39. The method of claim 30 wherein said gene is contained within a plasmid selected from the group consisting of pXL5 and pXL6.

40. A method of producing a vaccine for protection of a host against disease caused by infection with respiratory syncytial virus (RSV), which comprises:

isolating a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

operatively linking said first nucleotide sequence to at least one control sequence to produce a vector that will not replicate when introduced into the host to be protected, the control sequence directing expression of said RSV G protein when introduced to a host to produce an immune response to said RSV G protein,

operatively linking said first nucleotide sequence to a second nucleotide sequence to increase expression of said RSV G protein *in vivo* from the vector in the host, and

formulating said vector as a vaccine for *in vivo* administration to a host.

41. The method of claim 40 wherein said vector is selected from group consisting of pXL5 and pXL6.

42. A vaccine produced by the method of claim 40.

43. A method of determining the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising the steps of:

(a) immunizing a host with a vector tht will not replicate when introduced into the host to be protected to produce antibodies specific for the RSV G protein, said vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host,

(b) isolating the RSV G protein specific antibodies;

(c) contacting the sample with the isolated antibodies to produce complexes comprising any RSV G protein present in a sample and said isolated RSV G protein-specific antibodies; and

(d) determining the production of the complexes.

44. The method of claim 43 wherein said vector is selected from the group consisting of pXL5 and pXL6.

45. A diagnostic kit for detecting the presence of a respiratory syncytial virus (RSV) G protein in a sample, comprising:

(a) a vector that will not replicate when introduced into the host to be protected capable of generating antibodies specific for the RSV G protein when administered to a host, the vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;

(b) isolation means to isolate said RSV G protein-protein-specific antibodies;

(c) contacting means to contact the isolated RSV G specific antibodies with the sample to produce a complex comprising any RSV G protein in the sample and RSV G protein specific antibodies, and

(d) identifying to determine production of the complex.

46. The diagnostic kit of claim 45 wherein said vector is selected from the group consisting of pXL5 and pXL6.

47. A method for producing antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising:

(a) immunizing a host with an effective amount of a vector that will not replicate when instroduced into the host to be protected to produce RSV G-specific antibodies, said vector comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host; and

(b) isolating the RSV G-specific antibodies from the host.

48. A method of producing monoclonal antibodies specific for a G protein of respiratory syncytial virus (RSV) comprising the steps of:

(a) constructing a vector that will not replicate when introduced into the host to be protected comprising:

a first nucleotide sequence encoding a RSV G protein or a RSV G protein fragment that generates antibodies that specifically react with RSV G protein,

a promoter sequence operatively coupled to said first nucleotide sequence for expression of said RSV G protein in the host, and

a second nucleotide sequence located between said first nucleotide sequence and said promoter sequence to increase expression of said RSV G protein *in vivo* from said vector in the host;

(b) administering the vector to at least one mouse to produce at least one immunized mouse;

(c) removing B-lymphocytes from the at least one immunized mouse;

(d) fusing the B- lymphocytes from the at least one immunized mouse with myeloma cells, thereby producing hybridomas;

(e) cloning the hybridomas;

(f) selecting clones which produce anti-RSV G protein antibody;

(g) culturing the anti-RSV G protein antibody-producing clones; and then

(h) isolating anti-RSV G protein antibodies from the cultures.

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430 Rec'd PCT/PTO 14 JAN 2000

SEQUENCE LISTING

<110> LI, Xiaomao
SAMBHARA, Suryaprakash
KLEIN, Michel H.

<120> NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

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Ile Ile Ser Thr Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser
      50                  55                  60

Ala Asn His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr
 65                  70                  75                  80

Ser Gln Ile Lys Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln
                 85                  90                  95

Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr
            100                 105                 110

Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser Asn Leu Gln Pro
        115                 120                 125

Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser
    130                 135                 140

Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn
145                 150                 155                 160

Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys
                165                 170                 175

Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys
            180                 185                 190

Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe
        195                 200                 205

Lys Thr Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu
    210                 215                 220

Val Pro Thr Thr Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys
225                 230                 235                 240

Thr Asn Ile Thr Thr Thr Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro
                245                 250                 255

Lys Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr Ser Ser Glu Gly
            260                 265                 270

Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro Ser Gln
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Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln
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Ile Ser Phe Ser Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Thr Ile
         35                  40                  45

Leu Ala Ser Thr Thr Pro Gly Val Lys Ser Asn Leu Gln Pro Thr Thr
     50                  55                  60

Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln Pro Ser Lys Pro
 65                  70                  75                  80

Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn Asn Asp
                 85                  90                  95

Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn
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Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro
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09/462816

Restriction Map of the RSV G Gene

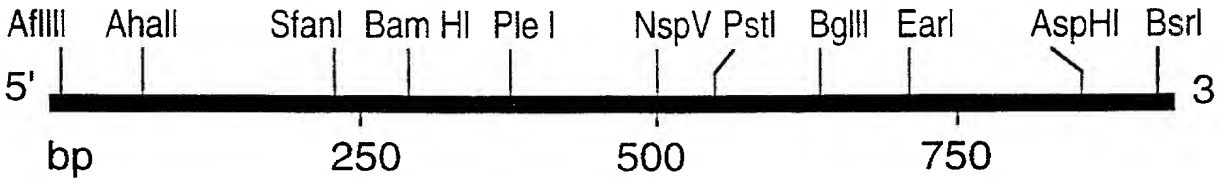

FIG.1

FIG.2A

```
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TGCAAAC ATG TCC AAA AAC AAG GAC CAA CGC ACC GCT AAG ACA CTA GAA AAG ACC
        Met Ser Lys Asn Lys Asp Gln Arg Thr Ala Lys Thr Leu Glu Lys Thr

          64         73         82         91        100        109
TGG GAC ACT CTC AAT CAT TTA TTA TTC ATA TCA TCG GGC TTA TAT AAG TTA AAT
Trp Asp Thr Leu Asn His Leu Leu Phe Ile Ser Ser Gly Leu Tyr Lys Leu Asn

         118        127        136        145        154        163
CTT AAA TCT GTA GCA CAA ATC ACA TTA TCC ATT CTG GCA ATG ATA ATC TCA ACT
Leu Lys Ser Val Ala Gln Ile Thr Leu Ser Ile Leu Ala Met Ile Ile Ser Thr

         172        181        190        199        208        217
TCA CTT ATA ATT ACA GCC ATC ATA TTC ATA GCC TCG GCA AAC CAC AAA GTC ACA
Ser Leu Ile Ile Thr Ala Ile Ile Phe Ile Ala Ser Ala Asn His Lys Val Thr

         226        235        244        253        262        271
CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG AAC ACA ACC CCA
Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys Asn Thr Thr Pro

         280        289        298        307        316        325
ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TCC AAT CTG TCT GAA
Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser Asn Leu Ser Glu
```

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FIG.2B

```
        334         343         352         361         370         379
ATT ACA TCA CAA ACC ACC ACC ATA CTA GCT TCA ACA ACA CCA GGA GTC AAG TCA
Ile Thr Ser Gln Thr Thr Thr Ile Leu Ala Ser Thr Thr Pro Gly Val Lys Ser

        388         397         406         415         424         433
AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACA ACC CAA ACA CAA
Asn Leu Gln Pro Thr Thr Val Lys Thr Lys Asn Thr Thr Thr Thr Gln Thr Gln

        442         451         460         469         478         487
CCC AGC AAG CCC ACT ACA AAA CAA CGC CAA AAC AAA CCA CCA AAC AAA CCC AAT
Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro Asn Lys Pro Asn

        496         505         514         523         532         541
AAT GAT TTT CAC TTC GAA GTG TTT AAC TTT GTA CCC TGC AGC ATA TGC AGC AAC
Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser Ile Cys Ser Asn

        550         559         568         577         586         595
AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA AAA CCA GGA AAG
Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys Lys Pro Gly Lys

        604         613         622         631         640         649
AAA ACC ACC ACC AAG CCT ACA AAA AAA CCA ACC TTC AAG ACA ACC AAA AAA GAT
Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr Thr Lys Lys Asp
```

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FIG.2C

```
        658         667         676         685         694         703
CTC AAA CCT CAA ACC ACT AAA CCA AAG GAA GTA CCC ACC ACC AAG CCC ACA GAA
Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr Lys Pro Thr Glu

        712         721         730         739         748         757
GAG CCA ACC ATC AAC ACC ACC AAA ACA AAC ATC ACA ACT ACA CTG CTC ACC AAC
Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr Leu Leu Thr Asn

        766         775         784         793         802         811
AAC ACC ACA GGA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC TTC CAC TCA ACC
Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr Phe His Ser Thr

        820         829         838         847         856         865
TCC TCC GAA GGC AAT CTA AGC CCT TCT CAA GTC TCC ACA ACA TCC GAG CAC CCA
Ser Ser Glu Gly Asn Leu Ser Pro Ser Gln Val Ser Thr Thr Ser Glu His Pro

        874         883         892         901         914
TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAGTTATTAA AAAAAAAAA
Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln  .
```

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FIG.3A

```
CAC AAA GTC ACA CTA ACA ACT GCA ATC ATA CAA GAT GCA ACA AGC CAG ATC AAG   54
His Lys Val Thr Leu Thr Thr Ala Ile Ile Gln Asp Ala Thr Ser Gln Ile Lys   18

AAC ACA ACC CCA ACA TAC CTC ACT CAG GAT CCT CAG CTT GGA ATC AGC TTC TCC  108
Asn Thr Thr Pro Thr Tyr Leu Thr Gln Asp Pro Gln Leu Gly Ile Ser Phe Ser   36

AAT CTG TCT GAA ATT ACA TCA CAA ACC ACC ACC ATA CTA GCT TCA ACA ACA CCA  162
Asn Leu Ser Glu Ile Thr Ser Gln Thr Thr Thr Ile Leu Ala Ser Thr Thr Pro   54

GGA GTC AAG TCA AAC CTG CAA CCC ACA ACA GTC AAG ACT AAA AAC ACA ACA ACA  216
Gly Val Lys Ser Asn Leu Gln Pro Thr Thr Val Lys Thr Lys Asn Thr Thr Thr   72

ACC CAA ACA CAA CCC AGC AAG CCC ACT ACA AAA CAA CGC CAA AAC AAA CCA CCA  270
Thr Gln Thr Gln Pro Ser Lys Pro Thr Thr Lys Gln Arg Gln Asn Lys Pro Pro   90

AAC AAA CCC AAT AAT GAT TTT CAC TTC GAA GTG TTT AAC TTT GTA CCC TGC AGC  324
Asn Lys Pro Asn Asn Asp Phe His Phe Glu Val Phe Asn Phe Val Pro Cys Ser  108

ATA TGC AGC AAC AAT CCA ACC TGC TGG GCT ATC TGC AAA AGA ATA CCA AAC AAA  378
Ile Cys Ser Asn Asn Pro Thr Cys Trp Ala Ile Cys Lys Arg Ile Pro Asn Lys  126

AAA CCA GGA AAG AAA ACC ACC ACC AAG CCT ACA AAA AAA CCA ACC TTC AAG ACA  432
Lys Pro Gly Lys Lys Thr Thr Thr Lys Pro Thr Lys Lys Pro Thr Phe Lys Thr  144
```

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FIG.3B

```
ACC AAA AAA GAT CTC AAA CCT CAA ACC ACT AAA CCA AAG GAA GTA CCC ACC ACC  486
Thr Lys Lys Asp Leu Lys Pro Gln Thr Thr Lys Pro Lys Glu Val Pro Thr Thr  162

AAG CCC ACA GAA GAG CCA ACC ATC AAC ACC ACC AAA ACA AAC ATC ACA ACT ACA  540
Lys Pro Thr Glu Glu Pro Thr Ile Asn Thr Thr Lys Thr Asn Ile Thr Thr Thr  180

CTG CTC ACC AAC AAC ACC ACA GGA AAT CCA AAA CTC ACA AGT CAA ATG GAA ACC  594
Leu Leu Thr Asn Asn Thr Thr Gly Asn Pro Lys Leu Thr Ser Gln Met Glu Thr  198

TTC CAC TCA ACC TCC TCC GAA GGC AAT CTA AGC CCT TCT CAA GTC TCC ACA ACA  648
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TCC GAG CAC CCA TCA CAA CCC TCA TCT CCA CCC AAC ACA ACA CGC CAG TAG      699
Ser Glu His Pro Ser Gln Pro Ser Ser Pro Pro Asn Thr Thr Arg Gln  .       232

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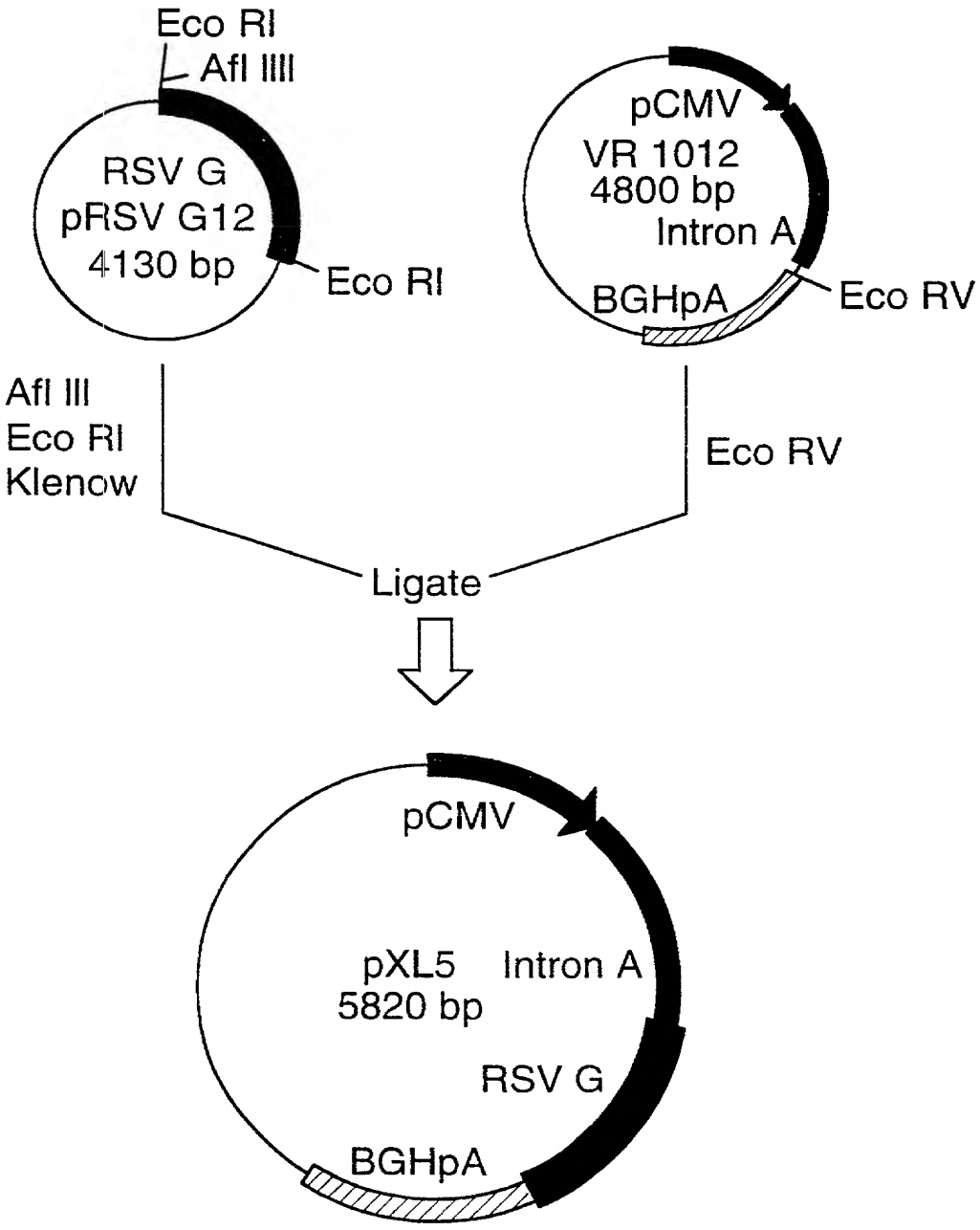
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pRSV G12
4130 bp
Eco RI
pCMV
VR 1012
4800 bp
Intron A
BGHpA
Eco RV
AfI III
Eco RI
Klenow
Eco RV
Ligate
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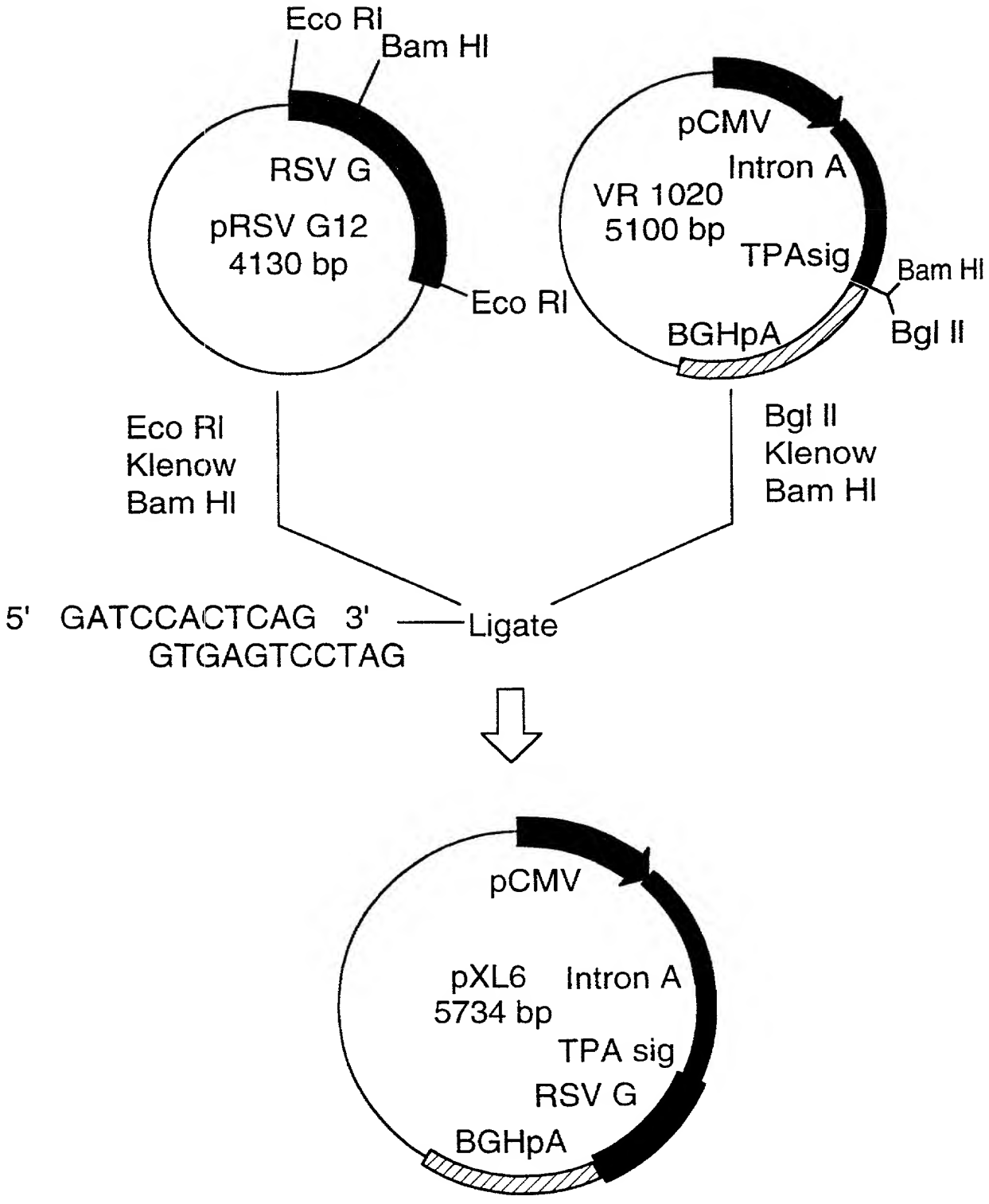
pXL5
5820 bp
Intron A
RSV G
BGHpA

FIG.4.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pRSV G12
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BGHpA
Eco RI
Klenow
Bam HI
Bgl II
Klenow
Bam HI
5' GATCCACTCAG 3'
GTGAGTCCTAG
Ligate
pCMV
pXL6
5734 bp
Intron A
TPA sig
RSV G
BGHpA

FIG.5

FIG.6A

10	20	30	40	50	60	70
TCGCGCGTTT	CGGTGATGAC	GGTGAAAACC	TCTGACACAT	GCAGCTCCCG	GAGACGGTCA	CAGCTTGTCT
80	90	100	110	120	130	140
GTAAGCGGAT	GCCGGGAGCA	GACAAGCCCG	TCAGGGCGCG	TCAGCGGGTG	TTGGCGGGTG	TCGGGGCTGG
150	160	170	180	190	200	210
CTTAACTATG	CGGCATCAGA	GCAGATTGTA	CTGAGAGTGC	ACCATATGCG	GTGTGAAATA	CCGCACAGAT
220	230	240	250	260	270	280
GCGTAAGGAG	AAAATACCGC	ATCAGATTGG	CTATTGGCCA	TTGCATACGT	TGTATCCATA	TCATAATATG
290	300	310	320	330	340	350
TACATTTATA	TTGGCTCATG	TCCAACATTA	CCGCCATGTT	GACATTGATT	ATTGACTAGT	TATTAATAGT
360	370	380	390	400	410	420
AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA	GTTCCGCGTT	ACATAACTTA	CGGTAAATGG
430	440	450	460	470	480	490
CCCGCCTGGC	TGACCGCCCA	ACGACCCCCG	CCCATTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG
500	510	520	530	540	550	560
CCAATAGGGA	CTTTCCATTG	ACGTCAATGG	GTGGAGTATT	TACGGTAAAC	TGCCCACTTG	GCAGTACATC

FIG.6B

```
570                 580                 590                 600                 610                 620                 630
AAGTGTATCA TATGCCAAGT ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC

640                 650                 660                 670                 680                 690                 700
CCAGTACATG ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG

710                 720                 730                 740                 750                 760                 770
GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTTTGACTC ACGGGGATTT CCAAGTCTCC

780                 790                 800                 810                 820                 830                 840
ACCCCATTGA CGTCAATGGG AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA

850                 860                 870                 880                 890                 900                 910
CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT ATATAAGCAG AGCTCGTTTA

920                 930                 940                 950                 960                 970                 980
GTGAACCGTC AGATCGCCTG GAGACGCCAT CCACGCTGTT TTGACCTCCA TAGAAGACAC CGGGACCGAT

990                 1000                1010                1020                1030                1040                1050
CCAGCCTCCG CGGCCGGGAA CGGTGCATTG GAACGCGGAT TCCCCGTGCC AAGAGTGACG TAAGTACCGC

1060                1070                1080                1090                1100                1110                1120
CTATAGACTC TATAGGCACA CCCCTTTGGC TCTTATGCAT GCTATACTGT TTTTGGCTTG GGGCCTATAC
```

FIG.6C

1130 1140 1150 1160 1170 1180 1190
ACCCCCGCTT CCTTATGCTA TAGGTGATGG TATAGCTTAG CCTATAGGTG TGGGTTATTG ACCATTATTG

1200 1210 1220 1230 1240 1250 1260
ACCACTCCCC TATTGGTGAC GATACTTTCC ATTACTAATC CATAACATGG CTCTTTGCCA CAACTATCTC

1270 1280 1290 1300 1310 1320 1330
TATTGGCTAT ATGCCAATAC TCTGTCCTTC AGAGACTGAC ACGGACTCTG TATTTTTACA GGATGGGGTC

1340 1350 1360 1370 1380 1390 1400
CCATTTATTA TTTACAAATT CACATATACA ACAACGCCGT CCCCCGTGCC CGCAGTTTTT ATTAAACATA

1410 1420 1430 1440 1450 1460 1470
GCGTGGGATC TCCACGCGAA TCTCGGGTAC GTGTTCCGGA CATGGGCTCT TCTCCGGTAG CGGCGGAGCT

1480 1490 1500 1510 1520 1530 1540
TCCACATCCG AGCCCTGGTC CCATGCCTCC AGCGGCTCAT GGTCGCTCGG CAGCTCCTTG CTCCTAACAG

1550 1560 1570 1580 1590 1600 1610
TGGAGGCCAG ACTTAGGCAC AGCACAATGC CCACCACCAC CAGTGTGCCG CACAAGGCCG TGGCGGTAGG

1620 1630 1640 1650 1660 1670 1680
GTATGTGTCT GAAAATGAGC GTGGAGATTG GGCTCGCACG GCTGACGCAG ATGGAAGACT TAAGGCAGCG

FIG.6D

1690 1700 1710 1720 1730 1740 1750
GCAGAAGAAG ATGCAGGCAG CTGAGTTGTT GTATTCTGAT AAGAGTCAGA GGTAACTCCC GTTGCGGTGC

1760 1770 1780 1790 1800 1810 1820
TGTTAACGGT GGAGGGCAGT GTAGTCTGAG CAGTACTCGT TGCTGCCGCG CGCGCCACCA GACATAATAG

1830 1840 1850 1860 1870 1880 1890
CTGACAGACT AACAGACTGT TCCTTTCCAT GGGTCTTTTC TGCAGTCACC GTCGTCGACA CGTGTGATCA

1900 1910 1920 1930 1940 1950 1960
GATATCGCGG CCGCTCTAGA CCAGGCGCCT GGATCCAGAT CTGCTGTGCC TTCTAGTTGC CAGCCATCTG

1970 1980 1990 2000 2010 2020 2030
TTGTTTGCCC CTCCCCCGTG CCTTCCTTGA CCCTGGAAGG TGCCACTCCC ACTGTCCTTT CCTAATAAAA

2040 2050 2060 2070 2080 2090 2100
TGAGGAAATT GCATCGCATT GTCTGAGTAG GTGTCATTCT ATTCTGGGGG GTGGGGTGGG GCAGGACAGC

2110 2120 2130 2140 2150 2160 2170
AAGGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG ATGCGGTGGG CTCTATGGGT ACCCAGGTGC

2180 2190 2200 2210 2220 2230 2240
TGAAGAATTG ACCCGGTTCC TCCTGGGCCA GAAAGAAGCA GGCACATCCC CTTCTCTGTG ACACACCCTG

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FIG.6E

2250 2260 2270 2280 2290 2300 2310
TCCACGCCCC TGGTTCTTAG TTCCAGCCCC ACTCATAGGA CACTCATAGC TCAGGAGGGC TCCGCCTTCA

2320 2330 2340 2350 2360 2370 2380
ATCCCACCCG CTAAAGTACT TGGAGCGGTC TCTCCCTCCC TCATCAGCCC ACCAAACCAA ACCTAGCCTC

2390 2400 2410 2420 2430 2440 2450
CAAGAGTGGG AAGAAATTAA AGCAAGATAG GCTATTAAGT GCAGAGGGAG AGAAAATGCC TCCAACATGT

2460 2470 2480 2490 2500 2510 2520
GAGGAAGTAA TGAGAGAAAT CATAGAATTT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT

2530 2540 2550 2560 2570 2580 2590
TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC

2600 2610 2620 2630 2640 2650 2660
GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT

2670 2680 2690 2700 2710 2720 2730
TTTTCCATAG GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC

2740 2750 2760 2770 2780 2790 2800
GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC GCTCTCCTGT TCCGACCCTG

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FIG.6F

2810 2820 2830 2840 2850 2860 2870
CCGCTTACCG GATACCTGTC CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA

2880 2890 2900 2910 2920 2930 2940
GGTATCTCAG TTCGGTGTAG GTCGTTCGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG TTCAGCCCGA

2950 2960 2970 2980 2990 3000 3010
CCGCTGCGCC TTATCCGGTA ACTATCGTCT TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA

3020 3030 3040 3050 3060 3070 3080
GCAGCCACTG GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC

3090 3100 3110 3120 3130 3140 3150
CTAACTACGG CTACACTAGA AGAACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA

3160 3170 3180 3190 3200 3210 3220
AAGAGTTGGT AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG

3230 3240 3250 3260 3270 3280 3290
CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT

3300 3310 3320 3330 3340 3350 3360
GGAACGAAAA CTGACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT

FIG.6G

```
3370       3380       3390       3400       3410       3420       3430
AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC

3440       3450       3460       3470       3480       3490       3500
TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCGGGGGGG

3510       3520       3530       3540       3550       3560       3570
GGGGGCGCTG AGGTCTGCCT CGTGAAGAAG GTGTTGCTGA CTCATACCAG GCCTGAATCG CCCCATCATC

3580       3590       3600       3610       3620       3630       3640
CAGCCAGAAA GTGAGGGAGC CACGGTTGAT GAGAGCTTTG TTGTAGGTGG ACCAGTTGGT GATTTTGAAC

3650       3660       3670       3680       3690       3700       3710
TTTTGCTTTG CCACGGAACG GTCTGCGTTG TCGGGAAGAT GCGTGATCTG ATCCTTCAAC TCAGCAAAAG

3720       3730       3740       3750       3760       3770       3780
TTCGATTTAT TCAACAAAGC CGCCGTCCCG TCAAGTCAGC GTAATGCTCT GCCAGTGTTA CAACCAATTA

3790       3800       3810       3820       3830       3840       3850
ACCAATTGTG ATTAGAAAAA CTCATCGAGC ATCAAATGAA ACTGCAATTT ATTCATATCA GGATTATCAA

3860       3870       3880       3890       3900       3910       3920
TACCATATTT TTGAAAAAGC CGTTTCTGTA ATGAAGGAGA AAACTCACCG AGGCAGTTCC ATAGGATGGC
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FIG.6H

3930	3940	3950	3960	3970	3980	3990
AATATCCTGG	TATCGGTCTG	CGATTCCGAC	TCGTCCAACA	TCAATACAAC	CTATTAATTT	CCCCTCGTCA
4000	4010	4020	4030	4040	4050	4060
AAAATAAGGT	TATCAAGTGA	GAAATCACCA	TGAGTGACGA	CTGAATCCGG	TGAGAATGGC	AAAAGCTTAT
4070	4080	4090	4100	4110	4120	4130
GCATTTCTTT	CCAGACTTGT	TCAACAGGCC	AGCCATTACG	CTCGTCATCA	AAATCACTCG	CATCAACCAA
4140	4150	4160	4170	4180	4190	4200
ACCGTTATTC	ATTCGTGATT	GCGCCTGAGC	GAGACGAAAT	ACGCGATCGC	TGTTAAAAGG	ACAATTACAA
4210	4220	4230	4240	4250	4260	4270
ACAGGAATCG	AATGCAACCG	GCGCAGGAAC	ACTGCCAGCG	CATCAACAAT	ATTTTCACCT	GAATCAGGAT
4280	4290	4300	4310	4320	4330	4340
ATTCTTCTAA	TACCTGGAAT	GCTGTTTTCC	CGGGGATCGC	AGTGGTGAGT	AACCATGCAT	CATCAGGAGT
4350	4360	4370	4380	4390	4400	4410
ACGGATAAAA	TGCTTGATGG	TCGGAAGAGG	CATAAATTCC	GTCAGCCAGT	TTAGTCTGAC	CATCTCATCT
4420	4430	4440	4450	4460	4470	4480
GTAACATCAT	TGGCAACGCT	ACCTTTGCCA	TGTTTCAGAA	ACAACTCTGG	CGCATCGGGC	TTCCCATACA

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FIG.6I

```
4490       4500       4510       4520       4530       4540       4550
ATCGATAGAT TGTCGCACCT GATTGCCCGA CATTATCGCG AGCCCATTTA TACCCATATA AATCAGCATC

4560       4570       4580       4590       4600       4610       4620
CATGTTGGAA TTTAATCGCG GCCTCGAGCA AGACGTTTCC CGTTGAATAT GGCTCATAAC GTTCCTTGTA

4630       4640       4650       4660       4670       4680       4690
TTACTGTTTA TGTAAGCAGA CAGTTTTATT GTTCATGATG ATATATTTTT ATCTTGTGCA ATGTAACATC

4700       4710       4720       4730       4740       4750       4760
AGAGATTTTG AGACACAACG TGGCTTTCCC CCCCCCCCCA TTATTGAAGC ATTTATCAGG GTTATTGTCT

4770       4780       4790       4800       4810       4820       4830
CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA

4840       4850       4860       4870       4880       4890       4900
AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAACCTA TAAAAATAGG CGTATCACGA

4910
GGCCCTTTCG TC
```

FIG.7

```
                10                  20                  30                  40                  50                  60                  70
CTGCAGTCAC CGTCGTCGAC CAGAGCTGAG ATCCTACAGG AGTCCAGGGC TGGAGAGAAA ACCTCTGCGA

                80                  90                 100                 110                 120                 130                 140
GGAAGGGAA GGAGCAAGCC GTGAATTTAA GGGACGCTGT GAAGCAATCA TGGATGCAAT GAAGAGAGGG

               150                 160                 170                 180
CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA GTCTTCGTTT CGCCCAGC
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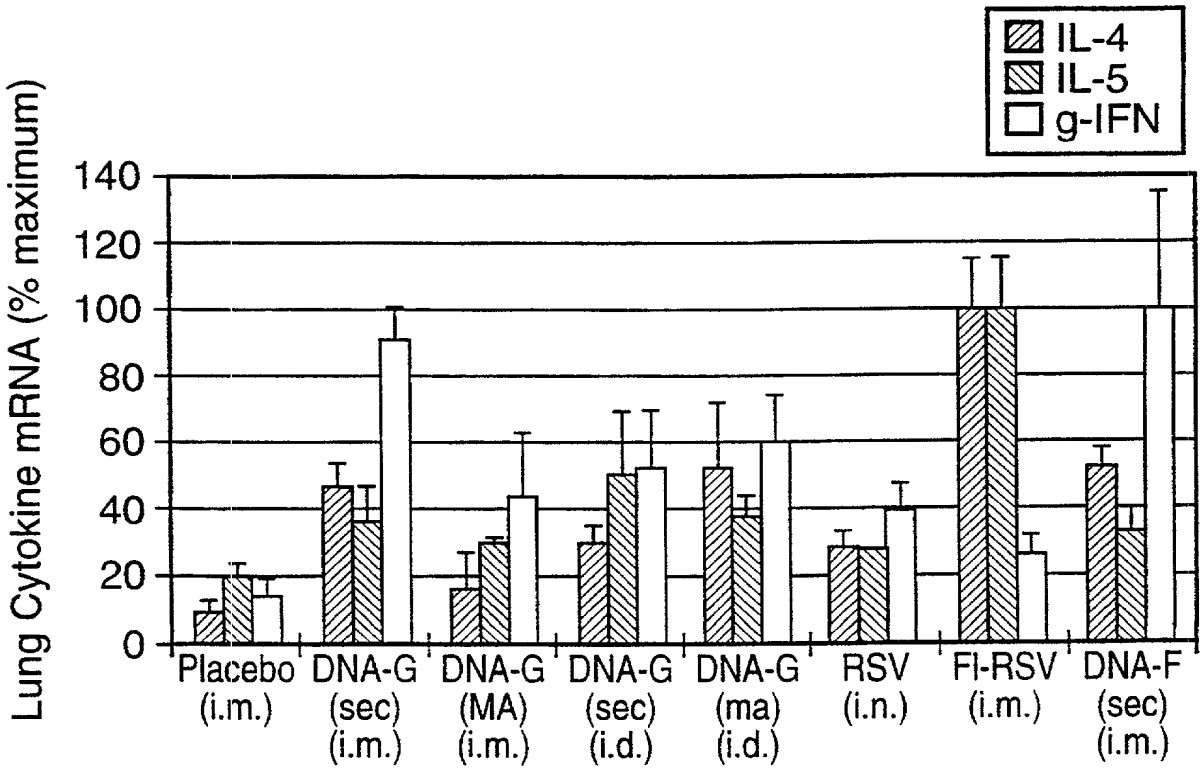
IL-4
IL-5
g-IFN
Lung Cytokine mRNA (% maximum)
140
120
100
80
60
40
20
0
Placebo (i.m.)
DNA-G (sec) (i.m.)
DNA-G (MA) (i.m.)
DNA-G (sec) (i.d.)
DNA-G (ma) (i.d.)
RSV (i.n.)
FI-RSV (i.m.)
DNA-F (sec) (i.m.)

FIG.8

OIPE
APR 0 5 2000
PATENT & TRADEMARK OFFICE

Docket No.
1038-1003 MIS:jb

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NUCLEIC ACID VACCINES ENCODING G PROTEIN OF RESPIRATORY SYNCYTIAL VIRUS

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **July 16, 1998** as United States Application No. or PCT International Application Number **PCT/CA98/00697**

and was amended on **September 9, 1999**

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

08/896,442	**July-18, 1997**	**Pending**
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
PCT/CA98/00697	**July 16, 1998**	
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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30

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Third inventor's signature Date Feb. 7/2020
Residence **Willowdale, Ontario, Canada.** CAX
Citizenship **Canadian**
Post Office Address **16 Munro Boulevard, Willowdale, Ontario, Canada, M2P 1B9.**

Full name of fourth inventor, if any
Fourth inventor's signature Date
Residence
Citizenship
Post Office Address

Full name of fifth inventor, if any
Fifth inventor's signature Date
Residence
Citizenship
Post Office Address

Full name of sixth inventor, if any
Sixth inventor's signature Date
Residence
Citizenship
Post Office Address